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Award Number: DAMD17-02-1-0363

TITLE: Identification of a Receptor for Tumor Suppressor HIN-1

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REPORT DATE: May 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
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20030902 105

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY</b> (Leave blank)		<b>2. REPORT DATE</b> May 2003	<b>3. REPORT TYPE AND DATES COVERED</b> Annual Summary (15 Apr 02 - 14 Apr 03)	
<b>4. TITLE AND SUBTITLE</b> Identification of a Receptor for Tumor Suppressor HIN-1			<b>5. FUNDING NUMBERS</b> DAMD17-02-1-0363	
<b>6. AUTHOR(S)</b> Dale A. Porter, Ph.D. Kornelia Polyak, M.D., Ph.D.				
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<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b>				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited				<b>12b. DISTRIBUTION CODE</b>
<b>13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)</b>  The goal of this project is to identify a receptor for the putative HIN-1 tumor suppressor gene. HIN-1 is a secreted protein therefore it is likely to exert its effect on cells through binding to a cell surface protein: the HIN-1 receptor (HIN-1R). Thus, in order to understand the function of HIN-1 we have to identify and characterize the HIN-1 receptor. This year we have made significant progress towards understanding the function of HIN-1 and the signaling pathways it may be involved in. Although the molecular identity of the HIN-1 receptor is still unknown, we have excluded several candidate receptors and signaling pathways and characterized the biochemical properties of the putative HIN-1R in further detail. Since the previously described expression cloning approach has not led to the identification of a HIN-1 receptor, we are trying additional approaches described here.				
<b>14. SUBJECT TERMS</b> gene expression, cell biology, expression cloning, receptor				<b>15. NUMBER OF PAGES</b> 5
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

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## Identification of a receptor for the HIN-1 tumor suppressor gene

### Introduction

The goal of this project is to identify a receptor for the putative HIN-1 tumor suppressor gene. HIN-1 is a secreted protein therefore it is likely to exert its effect on cells through binding to a cell surface protein: the HIN-1 receptor (HIN-1R). Thus, in order to understand the function of HIN-1 we have to identify and characterize the HIN-1 receptor.

This year we have made significant progress towards understanding the function of HIN-1 and the signaling pathways it may be involved in. Although the molecular identity of the HIN-1 receptor is still unknown, we have excluded several candidate receptors and signaling pathways and characterized the biochemical properties of the putative HIN-1R in further detail. Since the previously described expression cloning approach has not led to the identification of a HIN-1 receptor, we have tried several other alternative approaches listed below.

### Body

#### Expression Cloning:

Expression cloning experiments have been conducted to identify the HIN-1 receptor as described in the original proposal and outlined in the original statement of work. Unfortunately, despite extensive efforts screening ~1.25 million clones over more than a one year period, no HIN-1 receptor clone was identified using this technique. This possibility was not unforeseen, and, as described in the original proposal an alternative approach using  $^{125}\text{I}$  labeled recombinant HIN-1 has been initiated. Progress on this effort is summarized below.

#### Expression and purification of recombinant HIN-1 protein:

In order to further characterize the function of HIN-1 and to be able to perform ligand-binding studies to test candidate HIN-1 receptors, we have to generate HIN-1 protein in large quantities. Our progress has been hampered by difficulties associated with the production of pure recombinant HIN-1 protein in large quantities due to its unfavorable biochemical properties. In order to circumvent these problems we had to generate several different types of expression constructs and test the expression of HIN-1 in many different systems (various mammalian and bacterial cell types and in vitro protein production systems). We have developed a CHO mammalian cell system that enables to produce HIN-1 in the media of the cells, but the purification of non-denatured function HIN-1 protein turned out to be very inefficient. We have generated new constructs expressing HIN-1 protein fused to various other proteins (like growth hormone, glutathione transferase, hexahistidine and TEV protease cleavage site) in order to facilitate the purification of HIN-1. Among the several constructs and cell types tried, only the pSGV-TEV-HIN-1-T construct worked in CHO cells, although the yield of the protein was very low, and the pGEX4-HIN-1TT construct in bacterial cells. Using these two approaches we were able to generate some HIN-1 protein. Experiments conducted by Dr. Michele Parker have also demonstrated that recombinant HIN-1 protein purified from bacteria is functional in bio-assays. This protein will be labeled with  $^{125}\text{I}$  and used in binding and x-linking experiments to screen for and clone the HIN-1 receptor as described in the original proposal (alternative approach for aim 1).

#### Receptor binding studies:

Using AP-HIN-1 fusion protein we have performed several receptor-binding studies to better characterize the biochemical properties of the putative HIN-1 receptor. We found that trypsin and proteinase K reduce AP-HIN-1 binding to MCF10A cells, therefore, cell surface protein(s) are likely responsible for cell surface binding. Heparinase or salt wash pre-treatments led to increased AP-HIN-1 binding, while treatment of MCF10A cells with chloroquine did not appear to affect HIN-1 binding saturability, thus, the HIN-1 receptor is unlikely to be endocytic in nature. Treatment of human bronchial epithelial cells with retinoic acid led to up-regulation of HIN-1 and its putative receptor concomitant with the terminal differentiation of these cells, suggesting that HIN-1 may play an important role in this process.

#### Testing candidate HIN-1 receptors:

HIN-1 is distantly related to uteroglobin, another small secreted protein, and many of the functions of uteroglobin and HIN-1 appear to be related. Uteroglobin has been shown to bind to cubulin and megalin, two large cell surface and transmembrane proteins, respectively, suggesting that these may mediate uteroglobin's action. Due to the similarities between HIN-1 and uteroglobin, we analyzed if HIN-1 could also bind cubulin and megalin in collaboration with Dr. Moestrup (Denmark). Ligand binding studies performed using purified HIN-1, cubulin, and megalin proteins on a BIAcore chip concluded that HIN-1 does not appear to bind specifically to cubulin and megalin, thus, these proteins are not likely to serve as HIN-1 receptors.

#### Characterization of the HIN-1 signaling pathway:

As an additional alternative approach to understand HIN-1 function we have also analyzed the cellular response to HIN-1 using various genomics approaches. Specifically, we have developed an inducible HIN-1 expression system in breast cancer cells and determined the effect of HIN-1 expression on cell growth, invasion, and analyzed the gene expression and proteomics profiles of HIN-1 expressing cells. These assays revealed that HIN-1 dramatically suppresses cell growth and invasion. The analysis of the gene expression and proteomics pattern data is currently in progress. We hope that alterations of gene expression and proteomic pattern following HIN-1 expression will yield important information regarding the signaling pathways activated by HIN-1. This information then can be used for the identification of candidate receptors for HIN-1.

#### **Key Research Accomplishments:**

- ~1.25 million cDNA clones were screened but failed to identify a HIN-1 receptor.
- Several HIN-1 expression constructs have been generated, and some express a bio-active HIN-1 protein that will be used as a tool to screen for the HIN-1 receptor.
- Candidate receptors, cubulin and megalin, have been tested for HIN-1 binding activity and ruled out as potential HIN-1 receptors.
- Binding studies indicate that the HIN-1 receptor is a cell surface protein, is not likely to be endocytic in nature, and its expression is associated with a differentiated cell phenotype.

#### **Reportable Outcomes:**

- Recombinant, bio-active, HIN-1 protein can be purified in small amounts.
- Bio-informatics: New information indicates that HIN-1 belongs to a family of proteins called secretoglobins. Other secretoglobin family members have been shown to suppress cell growth consistent with a tumor suppressor role for HIN-1.

#### **Conclusions:**

In summary, we are using several alternative approaches for the identification of the HIN-1 receptor. We believe that using the combination of biochemical and genomic technologies we will be able to identify the receptor for the HIN-1 candidate tumor suppressor gene and further characterize the HIN-1 signaling pathway. Ongoing experiments in the lab continue to indicate that HIN-1 may play an important role as a tumor suppressor in breast cancer [1, 2].

#### **References:**

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